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Abstract
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of motor neurons in the brain and spinal cord. ALS shares pathobiological features with frontotemporal dementia (FTD) and indeed many patients show features of both diseases. It is now clear that many different genes and pathophysiological processes contribute to the disease, and that it will be necessary to understand this heterogeneity to find effective treatments. In this seminar we discuss current clinical and diagnostic approaches as well as scientific advances in the fields of genetics, disease modeling, biomarkers and therapeutic strategies.

Introduction
Amyotrophic lateral sclerosis (ALS) has traditionally been considered within the neuromuscular domain, despite the presence of selective degeneration of both upper and lower motor neurons. However, over the past decade, compelling clinical, imaging and neuropathologic data have emerged to indicate more extensive involvement of the neuro-axis than previously recognized. Detailed population-based phenotyping has now demonstrated that up to 50% of ALS patients develop cognitive and behavioral impairment, and that about 13% present with concomitant behavioral variant frontotemporal dementia (bv-FTD).[1,2] Pathology studies have demonstrated protein aggregation of TDP-43 in both ALS and FTD.[3]

Moreover, the discovery of hexanucleotide repeat expansions in the *Chromosome 9 open reading frame 72* gene (*C9orf72)* as the major genetic cause of ALS and FTD[4,5] proves beyond doubt that ALS and FTD, in at least some cases, constitute the phenotypic extremes of a spectrum of the same disorder (**Figure 1**),[6-10] placing ALS among neurodegenerative rather than neuromuscular diseases.

ALS has been traditionally divided into familial and sporadic forms. Over 30 different genes have been discovered to date in familial ALS,[11] leading to a redefinition of ALS as a clinically and genetically heterogeneous, multi-domain neurodegenerative syndrome of motor and extra-motor systems with multiple underlying pathophysiological mechanisms and different clinical sub-phenotypes.[9] This re-orientation will require the combined approaches of deep-phenotyping, neuroimaging, genomics and neuropathological evaluation if we are to further understand and eventually effectively treat this disease.

Epidemiology

The established incidence rate of ALS in populations of European extraction is 2.6-3.0/100,000,[12-15] with an overall lifetime risk of 1:350 for males and 1:400 for females.[16,17] Few true population-based studies are available from outside of Europe, but several studies support differences in the prevalence of ALS across African American, American First Nation, Hispanic, and non-Hispanic Caucasian groups. [18-23] There is also emerging evidence of lower incident and prevalent rates in populations of mixed ancestral origin, with differences in age of onset in admixed populations.[15,24-26] In populations of European ancestry, the median age of onset for sporadic ALS (SALS) is 65, whereas it is approximately 10 years earlier in mixed populations.[13,14,26-28] Although careful evaluation of population-based registers over time has not indicated substantial changes in the adjusted age-specific incidence, it is likely that the increased recognition of the ALS-FTD continuum has led to subtle shifts in the types of patients that are included on registers. This may partly explain the observed upward shift in the incidence of ALS, particularly in later life.[19,29,30] In most population-based studies, ALS is more common in males than females by a ratio of 1.2–1.5 to 1.[12-15] In contrast to Alzheimer's disease, the risk of developing ALS peaks between the ages of 50 and 75, and declines thereafter. Survival is highly variable, but on average patients die from respiratory failure 3-4 years after disease onset.[12-15]

Clinical presentations and diagnosis

ALS is characterized by progressive motor deficits that develop over the course of weeks to months. It may affect any voluntary muscle, which means the presentation is heterogeneous ranging from dysarthria to a foot drop (**Table 1**).[9] The motor neurons in the oculomotor nuclei, and Onuf's nucleus however appear to be relatively less vulnerable and therefore eye movement and sphincter control remain unaffected. On neurological examination both upper (UMN) and lower motor neuron (LMN) signs are present (**Figure 1**). The onset of the disease is focal in most patients and over time other regions of the body become affected. The pattern of disease progression (or spread) appears to be both local (within the same region, e.g. from hand to upper arm) as well as to neuro-anatomically linked regions (contra-lateral or rostral-caudal).[31]

The heterogeneous presentation and varying rates of progression render the diagnosis of ALS challenging. There is currently no diagnostic test that definitively demonstrates ALS, and the different differential diagnoses and investigations must therefore be tailored to each individual patient. The El Escorial or Awaji diagnostic criteria (**Table 3a**)[32,33], which are primarily used for research purposes, require a history of progressive weakness spreading within a region or to other regions (bulbar, cervical, thoracic or lumbar) with evidence of LMN (clinical or electrophysiological) and UMN (clinical) involvement and the exclusion of other disease processes that may otherwise explain the presentation.[32-35]
Patients are also often classified by site/pattern of onset or by degree of UMN/LMN involvement, which may have prognostic value (**Table 2**), but also helps to structure the differential diagnosis (DDx) and the diagnostic work-up (**Table 1**).[36] In **Figure 2** we provide a practical approach to a patient suspected of having ALS and an overview of the most useful ancillary investigations.

ALS variants
When UMN and LMN signs are clearly present in multiple regions, diagnosing ALS is relatively straightforward following exclusion of other possible diagnoses by imaging and neurophysiology. However, at disease onset UMN signs may be predominant and LMN involvement may only become evident at a later stage or vice versa. In these cases the differential diagnosis is more extensive and includes ALS variants, treatable ALS mimics and disorders with a more benign prognosis.[37] Recognizing these mimics and variants is therefore critical (**Figure 2**). A detailed discussion on the most common mimics is provided in the **supplementary material**.
The latest revision of the El Escorial diagnostic criteria contains restricted forms of ALS; progressive spinal muscular atrophy (PMA, exclusive LMN degeneration) and primary lateral sclerosis (PLS, exclusive UMN degeneration).[35] Whether these are indeed separate diseases or forms of ALS is a longstanding topic of debate, in particular for PMA. Autopsies of PMA patients have shown corticospinal tract involvement,[38] PMA patients may carry mutations in ALS-genes,[39] may have cognitive involvement[40] and patients in ALS-pedigrees have pure LMN phenotypes.[9]
Similarly, UMN degeneration, as occurs in PLS leads to progressive and disabling spasticity, but is rarely associated with respiratory failure. Therefore the prognosis of PLS is generally more benign (>10 years to normal lifespan) and important to diagnose.[41] The main challenge is to distinguish between UMN-predominant ALS, which usually progresses to a more generalized form of ALS within 4 years. Pure forms of hereditary spastic paraplegia (HSP) are an important diagnostic alternative to PLS. HSP is usually familial with young onset and is symmetrical, with limited or no involvement of the arms. Progression is usually slower in comparison to PLS and bulbar involvement is rare in HSP. Genetic testing for HSP-genes should be performed and in some cases the correct diagnosis only becomes evident through follow-up.[41-43]

Cognitive & behavioral changes in ALS
Cognitive change and behavioral change form an intrinsic component of some forms of ALS. The current approach is to first make a definitive diagnosis of ALS, and to subsequently screen for cognitive and behavioral changes. Studies show that 5-15% of ALS patients also have FTD

and in up to 50% cognitive or behavioral changes within the spectrum of FTD are present.[1,2,9,44] Similarly, 12.5% of bv-FTD patients develop ALS and mild motor neuron involvement is seen in approximately 40% of FTD patients.[45,46] The diagnostic criteria for FTD apply to ALS patients as they would to any other patient (**Table 3c)**.[47,48] Patients with cognitive or behavioral changes that do not fulfill formal diagnostic criteria can be grouped into 3 categories; ALS with behavioral impairment, ALS with executive impairment and ALS non-executive impairment (**Table 3b**).[49]

Many conventional neuropsychological tests require patients to be able to speak and write and therefore may not be suitable for use in ALS. A number of screening tools specifically designed for ALS are now available and include the ALS-Brief Cognitive Assessment (ALS-BCA)[50], ALS-Cognitive Behavioral Screen (ALS-CBS)[51], ALS-FTD-Q[52] and Edinburgh Cognitive and Behavioral ALS Screen (ECAS).[53] Patients who have abnormal scores on these screening tools should be referred for full neuropsychological assessment.
Apathy and loss of sympathy are the most common behavioral symptoms affecting approximately 10% of all patients.[53] Fluency, language, social cognition and executive function are the most commonly affected cognitive domains. Memory impairment may also be found, but rarely exists in isolation.[54] To date, only a limited number of longitudinal studies have been performed on cognition in ALS. Data suggests that patients without deficits at diagnosis remain unaffected and that cognitive decline in patients with non-executive impairment is slow or perhaps even stable. Executive dysfunction is associated with a more rapid disease progression.[55]
Recognizing cognitive and behavioral impairment is important as it is associated with mutations in specific genes (e.g. *C9orf72*, *TBK1*), more aggressive disease, non-compliance with treatment recommendations and increased care-giver burden.[50,51] Moreover, as impairment in capacity affects medico-legal decision-making, power of attorney should be discussed early in the disease in those with evidence of cognitive or behavioral changes.[56]

Pathophysiology
The mechanisms underlying neurodegeneration in ALS are still incompletely understood. A long list of cellular and molecular processes has been implicated and includes mitochondrial dysfunction, axonal transport, toxic protein aggregation, impaired protein degradation (proteasome and/or autophagy), prion-like spreading, excitotoxicity, lack of neurotrophic support from non-neuronal cells, dysfunction of non-neuronal cells, oxidative stress, hypermetabolism, inflammation, defects in RNA metabolism, RNA toxicity and others. Extensive literature providing convincing evidence for each of these mechanisms exists and has been reviewed elsewhere.[8,44] It is however possible that defects in some of these pathways are a secondary phenomenon and therefore genetics seems a logical starting point to disentangle this issue.
In 5-15% of patients ALS or FTD runs in the family (FALS)[9,57,58] and in these cases a single genetic defect is thought to cause disease. Functionally the majority of the 30 genes associated with FALS[11] can be grouped into 3 main pathophysiologic processes, namely RNA biology, protein turnover, and axonal transport suggesting that deficits in these pathways are causal.[8]
However, most patients have a negative family history, in which case the disease is (within the

caveats explained below) thought to be sporadic and to be caused by a combination of environmental and genetic risk factors.[17] In recent years multiple genetic risk factors for SALS have been identified. The search for environmental risk factors has however been less fruitful. Many case-control studies of exposure risks have been confounded by methodological errors and low power. High incidences of ALS have been recorded in Guam and the Kii Peninsula (Japan) and associations with cyanobacterial neurotoxins (BMAA) have been proposed, but never confirmed.[59-61]

Clustering of ALS has also been reported among Italian soccer players and American football players[62,63], and a number of detailed population-based, case-control studies have sought an association between intensive physical exercise and ALS, but with conflicting results.[64,65] It is possible the factors that determine an athletic disposition confer risk, rather than the actual exercise itself ("born to run" rather than "running to death"). Other environmental factors that have been associated with ALS include smoking, exposure to pesticides and organic toxins, and electromagnetic radiation.[17] With the exception of smoking[66], definitive evidence of risk remains to be established and will require large unbiased population-based case-control studies for confirmation.

The high degree of variability in phenotype and family history as well as the large number of genes, pathways and environmental risk factors that have been implicated seem to imply different mechanisms underlie neurodegeneration in different patients.

In fact, data from a recent study suggests that deficits in multiple pathways are required to develop ALS.[67] Interrogation of population-based registers demonstrated a log-linear relationship between incidence and age of onset, which similar to cancer, is consistent with a multistep model of disease. The number of steps required to cause disease can be estimated from the model as 6 steps. In this model each step represents a distinct pathophysiological process of which the last is the disease trigger. These findings emphasize the need to study genetic, environmental and lifestyle risk factors.[67] Although the multi-step model is still only a hypothesis, it is consistent with many features of ALS including, phenotypic variability, late-onset, non-penetrance, genetic pleiotropy and why the disease process cascades across the motor system rapidly after onset.

Although multiple mechanisms appear to be at play, abnormal aggregation of TDP-43 is the key pathological feature seen in nearly all ALS patients (with the exception of most *SOD1* and *FUS* cases), which suggests that altered function of this protein plays a crucial role in the disease.[3,68] Several hypotheses surround this topic. TDP-43 normally localizes to the nucleus where it has a function in transcription. In ALS TDP-43 is misfolded and aggregates in the cytosol and is thus mislocalized. Therefore nuclear loss-of-function resulting in transcription deficits has been suggested. TDP-43 aggregates may also acquire toxic properties through increased hydrophobicity and sequestration of essential cellular components, generation of oxidative species and proteasome inhibition.

Interestingly, there is mounting evidence that these aggregates might spread through a self-perpetuating or prion-like mechanism. Misfolded TDP-43, SOD1 and FUS are capable of forcing native protein into the misfolded configuration, which is perhaps aggravated under certain conditions (cell stress). These newly misfolded proteins (seeds) are in turn capable of misfolding

their native counterparts hereby initiating a cascade.[69-71] For SOD1 it has been shown that these seeds can spread to neighboring cells and within neuroanatomical pathways, which could be reflective of the clinically observed spread of disease.[71] Recently, cell-to-cell transmission via exosomes of dipeptide repeat proteins (DPRs) linked to *C9orf72* has also been reported.[72]

Recently another mechanism for disease spread was proposed. Several viral infections can cause motor neuron dysfunction (HIV, polio), but there is no evidence that ALS is due to viral infections. However, a substantial part of the human genome (8%) is comprised of viral sequence, which are remnants of infections that occurred in our distant ancestors and incorporated into the germline. The vast majority of this viral sequence has been rendered defective through the accumulation of non-sense mutations. Initial studies showed reverse transcriptase activity in the serum of ALS patients, and this was shown to be likely from activated endogenous retrovirus rather than acquired infection.[73-75]

A candidate virus was recently identified in a study demonstrating expression of the human endogenous retrovirus K (HERV-K) in the cortical and spinal neurons in a subpopulation of ALS patients, but not in healthy controls. The HERV-K genome encodes 3 genes, including one that encodes an envelope protein (*env*) which is selectively toxic to motor neurons in mouse models. Strikingly, expression of HERV-K genes is regulated by TDP-43. This raises the possibility that changes in TDP-43 may lead to the reactivation of inherited retroviral sequences resulting in the expression of HERV-K env and subsequent neurodegeneration.[76] Based on these data a clinical trial with HERV-K suppression has been initiated in the US (NCT02437110), and in Australia (NCT02868580).
Both the prion hypothesis and the viral reactivation theory pose interesting explanations for the manner in which the disease spreads after onset and could be the final step in the multi-step model.

Genetics of ALS
In approximately 60-80% of FALS patients a gene mutation of large effect (presumably pathogenic) can be identified, of which *C9orf72* (40%), *SOD1* (20%), *FUS* 1-5% and *TARBDP* (1-5%) are the most common.[11]
The genetics of SALS are less well understood. Twin studies have shown that the genetic contribution to SALS is considerable (61% (38%-78%)).[77,78] The latest genome-wide association study in ALS analyzed the genetic architecture of the disease by partitioning the explained heritability by allele frequency, and demonstrated that the remaining genetic risk factors are likely disproportionately to be rare variants (0.1–5%) with intermediate to large effects.[79] This implies that ALS is an oligogenic disease, which is distinct from many common disorders and neuropsychiatric conditions such as schizophrenia, which are highly polygenic (due to the additive effect of many common genetic polymorphisms with small effects).[80] An oligogenic model is consistent with the observation of incomplete penetrance in many ALS pedigrees, the reduced rate of ALS in admixed populations, and the presence of multiple ALS associated genes co-segregating with disease in some kindreds.[81-83] Heritability can also be obscured in small pedigrees (death resulting from other causes before the onset of ALS, loss of contact, etc.) causing familial cases to present as “apparently” sporadic.[84] This is reinforced by the finding

that approximately 10% of sporadic ALS cases have mutations in known FALS-genes and that first-degree relatives of sporadic patients are at an 8-fold higher risk of developing disease.[85] Rigid dichotomizing ALS into familial and sporadic disease can now be considered an over-simplification, as all of the evidence points towards similarities in genetic architectures between familial and sporadic disease.

Moreover, it is also increasingly recognized that ALS genes may be pleiotropic, meaning that they are involved in multiple phenotypes. The most established example of pleiotropy is *C9orf72,* which is clearly linked to ALS and FTD, but also to PLS, PMA, Parkinsonism, Huntington phenocopies, Alzheimer's disease, corticobasal degeneration, schizophrenia, psychosis and bipolar disorder.[10] Other examples of pleiotropy are repeat expansions in *ATXN2* gene (in which pure-CAG expansions cause spinocerebellar ataxia type 2, but intermediate-length interrupted repeats are risk factors for ALS and parkinsonism).[86,87] Similarly, rare genetic variation in *ANG* is a risk factor for ALS and parkinsonism[88,89] and mutations in *hnRNPA1*, *hnRNPA2b1, SQSTM1* and *VCP* have been reported in pedigrees with a heterogeneous phentoype (also known as multisystem proteinopathy) that includes ALS, FTD, IBM and Paget's disease of the bone.[90-92] Other genes, including *Matr3*, *CHCHD10* and *SQSTM1* have also been implicated in myopathies.[93-95]

Considering the genetic architecture of ALS, it is likely that whole genome sequencing of large numbers of patients and controls will be required to fully understand the genetics of this disease. An international whole genome-sequencing project was initiated in 2012 with the goal of sequencing the complete genomes of 15,000 ALS cases and 7,500 controls (www.ProjectMine.com) and is estimated to be completed by the end of 2017.

Notwithstanding the advances in our understanding of ALS from a genomic perspective, substantial dilemmas remain from a clinical perspective. While some ALS mutations are directly pathogenic, this has not been demonstrated for many reported variants. For instance, over 150 mutations have been reported in *SOD1*, but irrefutable evidence for direct pathogenicity is only available for a few mutations (e.g. p.A5V, homozygous p.D91A).[96,97] Similarly, initial studies suggested that *C9orf72* is fully penetrant by the age of 80, but there is now a growing number of reports of asymptomatic *C9orf72* expansion carriers of advanced age, and penetrance estimation using statistical methods suggests this mutation has only moderate penetrance (http://alsod.iop.kcl.ac.uk/misc/penetrance.aspx).[84]

Non-penetrance and genetic pleiotropy in ALS is incompletely understood and *C9orf72* perhaps best illustrates the complexity of this topic. Disease severity and phenotype appear to be dependent on the size of the repeat expansion (which may vary between cell types within an individual (mosaicism)), methylation status of the promotor and the expansion itself as well as the presence of genetic variation in other genes (e.g. *TMEM106b*, *ATXN2* and others).[10,98-102]

Providing genetic counseling to ALS patients and their relatives is becoming increasingly challenging. There is a growing realization among patients in the Internet era that their disease may be genetic and the "right to know" is a basic principle of human clinical genetics recognized by most international regulatory statements and legislation.[103,104] However, given the complexity of the subject, opinions regarding genetic testing differ.[105-107] Recently a group of neurologists and clinical geneticists proposed guidelines for genetic testing in ALS, in which they suggest that genetic testing should be offered to all patients with a first or second degree relative with ALS or FTD and the option of genetic testing should be discussed

with all other patients.[105] Counseling should be provided by individuals with an up-to-date understanding of ALS genetics, who are willing to take responsibility for the interpretation of the results. It would seem advisable to limit testing to those genes for which there is strong evidence for causality; *C9orf72*, *TARBDP*, *FUS* and *SOD1*, and the local geographic distribution of known causative mutations should be taken into account.[108]

From genes to biology

For a long time *SOD1* was the only known gene for ALS and transgenic *SOD1*-mice were the only available ALS disease model.[109] Although this model recapitulates several aspects of ALS, it is probably not representative for most forms of ALS because pathological TDP-43 accumulation is not present (**Table 4**). This may be a possible explanation why translation of therapeutic approaches developed in this model to human patients has been difficult.[110]

Recent genetic discoveries and advances in molecular biology have facilitated the generation of multiple novel ALS models for different genes (e.g. *TARDBP*, *FUS*, *C9orf72*, *VAPB, VCP*) in different species (*C. elegans*, *Drosophila*, zebrafish, mouse, rat).[109,111-115] Similar to *SOD1*-mice these new models often do not display all features characteristic for the ALS patients carrying corresponding mutations, but they have proven to be extremely valuable for understanding the effects of gene mutations at the molecular, cellular and systems levels. With ongoing discovery of ALS genes and the development of powerful genome editing such as CRISPR/CAS many more ALS models are expected to be generated in the coming years. In addition, to animal models, stem cell-based cellular models have become increasingly important in ALS research. The ability to convert somatic cells from humans, e.g. skin fibroblasts, into induced pluripotent stem cells (iPSCs) has revolutionized research into human disease.[116] Several studies have already used iPSC technology to generate patient-derived motor neurons and employed these cultures to detect cellular defects such as impaired neurotransmission, cell death and altered neuronal morphology.[117] Given the fact that the (epi-)genetic makeup of patients is highly preserved in iPSC-generated human motor neurons these cultures are viewed as promising models for future screening of therapeutic compounds**.**[117]

Using these models and novel techniques considerable advances have been made in the understanding of mechanisms underpinning *C9orf72* pathophysiology. Three different, but not mutually exclusive, mechanisms have now been proposed. The first proposed mechanism is haplo-insufficiency, which is supported by decreased C9ORF72 mRNA and protein expression in brain tissue of patients.[4] Secondly, as in other repeat expansion disorders, C9ORF72 RNA may accumulate in so-called RNA foci, which traps other RNA molecules or RNA binding proteins and thereby affects RNA biology.[4] Thirdly, ATG-independent RAN translation has been shown. Based on the frame and the direction in which the repeat is read, it codes for several short dipeptide repeat proteins (DPRs), which appear to have toxic properties.[118,119] Interestingly, DPRs can be measured in CSF and may be a useful biomarker either diagnostically or as an outcome measure in clinical trials.

Current and future treatments

Presently, Riluzole is the only widely available drug that has been shown to prolong survival in ALS. The most recent Cochrane review showed that there is a 9% gain in the probability of

surviving one year for patients on Riluzole compared to the placebo-group, corresponding to an increase in median survival from 11.8 to 14.8 months.[120,121]
Recently, Edaravone (a free radical scavenger) was approved for the treatment of ALS in Japan, but has not been approved elsewhere. The results from the trial (NCT01492686) demonstrating efficacy however remain to be published. Preliminary reports suggest that Edaravone significantly slows functional decline over a 24-week period compared to placebo in a subcohort of patients characterized by recent disease onset and relative preservation of respiratory function.
Nuedexta has been shown to be effective for treating pseudobulbar affect (uncontrollable laughing or crying) in ALS and there are anecdotal reports of improvement in speech and swallowing.[122] It is not available outside of the USA, although initially marketing authorization for Europe was granted. It was however redrawn at the request of the marketing authorization holder, apparently based on commercial considerations.
Differences in drug availability and inconsistencies in decisions from regulatory agencies are very frustrating to ALS patients, because they feel they are being denied potentially effective treatments. Harmonization of criteria for approval of treatments for lethal diseases, such as ALS, between regulatory agencies would therefore be highly desirable.

Precision Medicine
We now recognize ALS as a syndrome rather than a single disease entity and that therefore different pathophysiological mechanisms may be at play in different subtypes. While these mechanisms may converge on shared final common pathways resulting in recognizable clinical sub-phenotypes, it is likely that different subtypes of ALS will respond to different disease modifying therapies. The greatest challenge in ALS will be to unravel the heterogeneity and recategorize patients according to (genetic) subgroup or most relevant pathophysiological feature (**Figure 3**), which will facilitate the development of targeted treatments and move the field towards precision medicine.
This would dramatically alter the way trials are conducted. Inclusion criteria would be based on genetics or other biomarkers. This will require large-scale international harmonization of subtype classification to permit the enrolment of sufficient numbers of patients for such trials.
The first steps towards precision medicine in ALS have already been taken, as a successful phase 1 study with *SOD1* antisense oligonucleotides has been performed and a new phase 1 trial with a potentially more effective oligo is under way.[124] Many research groups are working on gene-silencing therapies for *C9orf72* through antisense oligonucleotides, viral delivery or si-RNA and small molecules. Initially C9ORF72 knockout models did not demonstrate any phenotype, suggesting that this would be a safe strategy.[125] However, recent studies have demonstrated that the complete knockdown of C9ORF72 has profound consequences and leads to severe immune system dysfunction and neoplastic events.[126] Therefore, it seems critical that selective knock-down of the expanded allele is achieved.
A recent study in Alzheimer's disease showed that the monoclonal antibody, Aducanumab, selective targets aggregated Aβ, lowers soluble and insoluble Aβ in a dose-dependent manner and that monthly intravenous infusions slow memory decline in patients with prodromal or mild AD.[127] Based on this approach, one could contemplate targeting TDP-43 in a similar

fashion. TDP-43 levels are however tightly regulated and overexpression and knock-down could be detrimental and therefore not as straightforward as may seem.
Pioneering work is also being undertaken with the transplantation of neural stem cells in the spinal cord of ALS patients and can be done safely. Results from efficacy trials are eagerly awaited.[128,129]

Symptomatic Therapies
In the absence of effective pharmacological treatments, symptomatic interventions and supportive care remain the cornerstone of ALS-management.[130-132] Several of these symptomatic therapies are associated with a clear survival benefit, whereas others provide symptom relief and therefore positively influence quality of life.

Symptomatic therapies with survival benefit:
- Studies have shown that care is most effective and positively impacts survival when delivered by a multi-disciplinary team, including physiotherapists, occupational therapists, speech therapists, respiratory physicians, dieticians, gastroenterologists, social workers, family physicians, neurologists and rehabilitation physicians.[133,134]
- Weight loss is commonly seen in ALS as the disease progresses and is multifactorial in nature (loss of muscle tissue, hypermetabolism, difficulties eating (swallowing or shortness of breath) or decreased appetite). Multiple studies have shown that prevention of malnutrition improves survival and quality of life.[135] Guidelines recommend patients to undergo gastrostomy to enable enteral feeding and hereby sustain nutrition and medication intake when 10% of body weight has been lost. However, a recent study showed that the majority of patients who had lost more than 10% of their premorbid body weight failed to regain weight after following gastrostomy and even continued to lose weight. The authors therefore suggest that placement of a gastrostomy tube is most effective an earlier stage (5% weight loss).(REF: Stavroulakis, T. et al. The impact of gastrostomy in motor neurone disease: challenges and benefits from a patient and carer perspective. BMJ Support. Palliat. Care 6, 52–59 (2016))
- Non-invasive ventilation (NIV) prolongs survival with an effect size greater than riluzole.[136] The use NIV at night (and during daytime if required) is associated an increase in median survival of 7 months and also improves quality of life.[136] However, NIV-use requires significant effort from patients, carers and respiratory physicians and is therefore not be feasible for all patients, particularly those with cognitive impairment or severe bulbar problems. Results from a cohort study including 929 patients suggest NIV also benefits survival in bulbar-onset patients and that a trial of NIV should be offered to all patients, even when likely to be poorly tolerated.[137]
Considering the challenges associated with NIV, alternative strategies for maintaining/supporting respiration are desirable. Diaphragm pacing or phrenic stimulation was approved as treatment for respiratory failure based on two studies showing that implantation appears safe and better survival in implanted patients with NIV compared to historical controls on NIV only (37.5 versus 21.4 months respectively).[138,139] However, two recent randomized-controlled trials contradict this finding. In fact, both studies observed a significant excess of mortality in the implanted patients with NIV compared to those on NIV-only. These findings caused both trials to be stopped prematurely. Although the mechanism underlying a potentially

harmful effect of diaphragm pacing is not clear, the use of diaphragmatic pacing **is NOT (?)** recommended as a routine treatment for patients with ALS in respiratory failure.[140,141]

Treatments with symptomatic benefit:
Over the course of the disease many signs and symptom may develop such as excess salivation, emotional lability, dropped head, frozen shoulder, pain, cramps, spasticity and others. Expert consensus guidelines for the management of these aspects of ALS are available and have been reviewed elsewhere.

The importance of biomarkers
The identification of reliable biomarkers is a high priority in ALS.[145] Diagnostic biomarkers could reduce diagnostic delay (presently 9-12 months) and would facilitate early initiation of treatment, which is likely when it is most effective in a neurodegenerative disease.
- *Current measures of disease progression:* The primary outcome measure in ALS trials is survival and/or rate of decline on the ALS Functional Rating Scale–revised (ALS-FRS-R).[146,147] Although robust, a considerable amount of time needs to pass before these outcome measures become informative, resulting in lengthy and expensive trials. Early and reliable biomarkers could potentially accelerate trials and therefore the quest for an effective treatment for ALS. Decline in muscle strength and respiratory function have extensively been studied as markers of disease progression. There are several ways to measure muscle strength[148-150], of which hand held dynamometry is probably the preferred method in the field presently as it can be performed rapidly, is cheap, quantitative, reliable and a reproducible measure of decline in ALS.[151] Similarly different measures exist for respiratory function, including vital capacity, sniff nasal inspiratory pressure and maximal inspiratory pressure. Differences of opinion exist on which is the best measure and all are commonly used.
Although muscle strength and respiratory function are informative markers, they do not represent early changes in ALS. Clinical weakness only becomes apparent after a substantial number of motor neurons are lost and is initially compensated for by reinnervation. Respiratory dysfunction develops late in the disease in most patients. Therefore more accurate biomarkers of disease progression are urgently needed. Moreover, considering ALS affects lower and upper motor neurons, but also other brain areas (e.g. frontal and temporal lobes), different biomarkers might be required for different aspects of the disease.[152]
- *LMN biomarkers:* LMN loss prior to the development of clinical weakness can be assessed using different electrodiagnostic methods.[153] Nerve conduction studies show that the compound muscle action potential (CMAP) amplitude declines over time and is sensitive to disease progression. The CMAP amplitude is however also influenced by reinnervation and therefore does not allow quantification of LMN loss. Motor unit estimation (MUNE)[154] and Motor Unit Index (MUNIX)[155] are neurophysiological methods that aim to estimate the number of remaining motor units innervating a muscle by dividing the maximal CMAP by the average surface single motor unit action potential or from the inference pattern on surface EMG and maximal CMAP at different grades of voluntary muscle contraction. The advantage of MUNE and MUNIX is that they provide an estimation of the number of motor units, although it must be noted that these results are highly correlated with the CMAP. Other potential biomarkers for LMN loss under investigation include nerve excitability, electrical impedance myography and

muscle ultra-sound. [156-158] All techniques have their own pros and cons with regards to reproducibility, availability and complexity. Currently there is no single preferred method.
- *UMN biomarkers:* Transcranial magnetic stimulation (TMS) is a non-imaging based technique that can be used to measure UMN dysfunction. A magnetic coil is used to excite neurons in the underlying motor cortex and subsequently motor evoked potentials are recorded over a contralateral hand muscle. TMS improves the sensitivity of ALS diagnosis, but has the disadvantage that it is technically challenging in patients with severe hand muscle atrophy.[165]
- *Imaging biomarkers:* UMN loss may be difficult to detect clinically as it may be masked by LMN loss and validated clinical UMN scores are lacking. Other measures are therefore desirable. Different imaging techniques have been widely applied to study UMN loss. MRI is able to distinguish ALS cases from mimics and healthy controls at group level, and some studies suggest that cortical thinning of the primary motor cortex is a sensitive diagnostic marker at individual patient level.[159,160] A recent meta-analysis on DT MRI diagnostic accuracy in ALS reported a pooled sensitivity of 65% and specificity of 67%.[161]Also resting state fMRI studies in ALS seem to have a good sensitivity and specificity when assessed by machine learning methodologies.[162]
A role of ^{18}F-FDG-PET as a diagnostic biomarker was suggested by two recent large studies that showed motor and extra-motor hypometabolism as well as of hypermetabolism in brainstem and medial temporal cortex with an overall accuracy in discriminating ALS patients from controls of 93%.[163,164]
- *Wet biomarkers:* Blood or CSF biomarkers would be equally attractive and at present the most interesting candidates are neurofilaments, which are major structural proteins in neurons that are released following neuronal damage. CSF neurofilament light chain (NfL) and phosphorylated heavy chain levels have a good sensitivity (77% and 83%) and specificity (85% and 77%) in differentiating ALS from mimics and show moderate correlation with progression.[169] Serum NfL have >90% sensitivity and specificity in separating ALS patients from healthy controls, but data on comparison with ALS mimics are not available.[170] Moreover, the immunoreactivity to plasma neurofilament light chain changes are related to ALS clinical staging, indicating that this biomarker may be also sensitive to disease progression.[171]
- *Biomarkers of disease progression:* Longitudinal measurements of cognition and behaviour could potentially detect changes over time and therefore serve as a marker for spread of the disease to other brain areas (frontal and temporal lobes). Considering aggregation of TDP-43 is the pathological hallmark of ALS, it stands to the reason that being able to image this in-vivo, as is possible with amyloid and Tau, could be a powerful biomarker for all aspects of the disease.[166] At present this is not possible, but efforts are underway.
Although all of the techniques mentioned show promise, they all require equipment, time, expertise and/or substantial resources. The ideal biomarker should be possible to measure simply and reliably. A potential approach to this end is to assess disease progression through staging, which would allow the use of time from one stage to another instead of time to death as an outcome measure. Several staging systems exist and indeed correlate with existing measures such as the ALS-FRS-R.[167,168]

Conclusions

We now recognize that ALS is a heterogeneous syndrome that shares pathobiological features with FTD. The rapid pace of gene discovery has facilitated the study of the molecular biology of ALS. There are now many different genetic models of ALS and studying these has uncovered many new potential therapeutic targets. There is a sense of optimism in the field that this progress will lead to the so urgently needed treatment for ALS.

Search strategy and selection criteria

We searched Pubmed and Google Scholar (1966, to April 2016) and the Cochrane Library using the search terms "amyotrophic lateral sclerosis" or "motor neuron disease" or "frontotemporal dementia" in combination with "diagnosis", "epidemiology", "frontotemporal dementia", "imaging", "neurophysiology", "management", "genetics", "biomarkers", "treatment", "C9orf72", and "neuroprotection". Further articles were included from reference lists and review articles. Abstracts and reports from relevant meetings were also included. The final reference list was generated on the basis of originality and relevance to the topics covered in this Seminar. Emphasis was placed on publications from the past 5 years, but did not exclude commonly referenced and highly regarded older publications.

Contributors

MAvE & LHvdB did the literature review, coordinated authors' writing, revision, and editing, wrote the first draft, prepared figures, and finalized the manuscript. OH did the literature search and contributed to sections on epidemiology, cognition and was involved in drafting the revision and editing of the final version of the manuscript. AC did the literature search and contributed to sections on epidemiology, cognition and biomarkers. AAC did the literature search and contributed to the sections on viruses, genetics and biomarkers. RJP and JHV did the literature search and contributed to sections on genetics, from genes to biology and the *C9orf72* panel. All authors were involved in critical revision of the manuscript.

Conflicts of interest

MAvE serves on the Motor Neurone Disease Association biomedical research advisory panel, has consulted for Biogen and has received travel grants from Baxalta and funding sources include the Netherlands Organization for Health Research and Development (Veni scheme), The Thierry Latran Foundation, the ALS Foundation Netherlands LHvdB received travel grants and consultancy fees from Baxalta, and serves on the advisory board for Biogen and Cytokinetics. AAC serves on the MND Association genetics and epidemiology data access committee, and has consulted for Biogen, OrionPharma, Cytokinetics and Mitsubishi-Tanabe. AC serves on the advisory board for Biogen, Cytokinetics and Mitsubishi Tanabe.

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